Ultracentrifugation of Barley β-Amylases

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We have recently described the fractionation of the β-amylases of barley grain by exclusion chromatography on Sephadex G-100 and G-75 columns.[1,2] There are at least four immunochemically identical β-amylases of different molecular size in barley.[3] The fractions of the free β-amylase were called A_1-A_4 from the smallest to the largest and the bound β-amylase was called A_B. With the object of gaining more information about the heterogeneity of this enzyme an ultracentrifugal examination was also made of the β-amylase fractions obtained by exclusion chromatography.

Experimental. The preparation of β-amylases of different molecular size was performed by salting-out with ammonium sulphate and exclusion chromatography, as described earlier.[4] According to this method, the free β-amylases are extracted with distilled water from acetone-treated barley grist. The bound enzyme is extracted, after several washings with salt solutions, with a thioglycolic acid solution. The largest free β-amylase, A_4, was precipitated by salting-out with ammonium sulphate at 18 % saturation. After precipitation, fraction A_4 was subjected to chromatography on Sephadex G-100. The result of rechromatography of this fraction is shown in Fig. 1. A_1 and A_2 were precipitated together by ammonium sulphate between 23 and 30 % saturation and subjected to chromatography on the same column as A_4. The result is shown in Fig. 1.

The bound enzymes were also prepared according to the same method.[4] There was only a small amount of the larger, bound β-amylase, A_{B2}; therefore the A_{B2}-peaks from several runs were collected and rerun together. The results are shown in Fig. 2.

All the separated β-amylase fractions were collected after the final chromatography according to the drawings in Figs. 1 and 2, and dialysed overnight against a phosphate buffer (0.2 M sodium chloride, 0.01 M phosphate, pH 7.3, ionic strength 0.23).

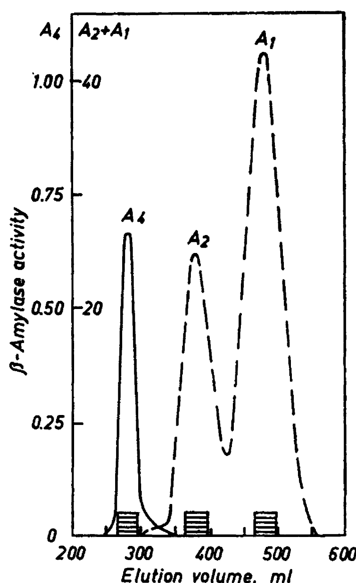

Fig. 1. Exclusion chromatography of free β-Amylases of barley on a Sephadex G-100 column (4 × 67 cm) after previous salting-out. ——— = fraction precipitated by ammonium sulphate at 0−18 % saturation. – – – = fraction precipitated by ammonium sulphate at 23−30 % saturation. The column was packed in 0.010 M sodium phosphate buffer of pH 7.5, containing 2.5 % sodium chloride. β-Amylase activity = mg maltose/min. × ml.

Every dialysed β-amylase fraction was examined in the Spinco model E analytical ultracentrifuge at 59 780 rpm at 19−21°C, a wedge cell being used for the simultaneous running of two samples. Every fraction was studied at two concentrations, using the original concentration and a dilution of 1:2. The sedimentation coefficients of the fractions were determined according to conventional methods. The observed sedimentation coefficient was corrected to the value it would have in a solvent having the density and viscosity of water at 20°C. This corrected value ($s_{20,w}$) was extrapolated to infinite dilution for obtaining $s°_{20,w}$. The sedimentation coefficients are given in Svedberg units (S).

Results and discussion. The sedimentation coefficients at infinite dilution ($s°_{20,w}$)

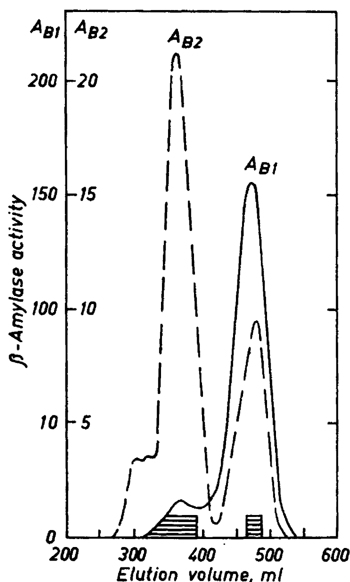

Fig. 2. Exclusion chromatography of bound β-Amylase of barley on a Sephadex G-100 column.
——— = first chromatography of the bound enzyme.
– – – = rechromatography of the collected A_{B2}-peak from the previous runs. The runs were made on the same column as in Fig. 1. β-Amylase activity = mg maltose/min × ml.

Table 1. Sedimentation coefficients of free and bound barley β-amylase fractions. The sedimentation coefficients of the main boundaries are in bold face.

Fraction of β-amylase		$s^{\circ}_{20,w}$		Number of boundaries
Free	A_1	**4.6** S		One
	A_2	4.6 S	**6.8** S	Two
	A_4	.. [a]		One (fast-diffusing)
Bound	A_{B1}	**4.6** S		One
	A_{B2}	.. [a]	**6.2** S	Two

[a] $s^{\circ}_{20,w}$ of these boundaries could not be determined.

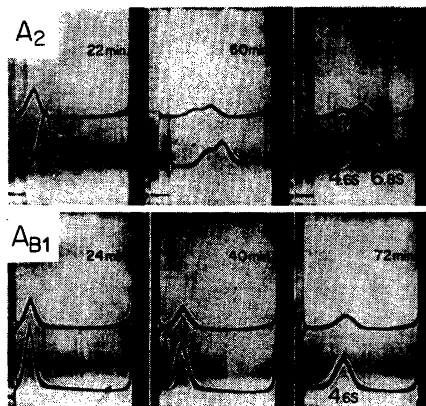

Fig. 3. Sedimentation patterns of barley β-amylase fractions A_2 and A_{B1}. The times given are minutes after reaching full speed.

of free and bound β-amylase fractions are seen in Table 1.

The boundaries of fractions A_1 and A_{B1} moved across the cell during the run as one symmetrical peak, whereas the boundaries of the fractions A_2 and A_{B2} dissolved in two peaks during the run (Fig. 3). The amount of the slower peak in both these cases was smaller than that of the faster peak. It was not possible to determine the sedimentation coefficient of the slower peak of fraction A_{B2} because it was only partly separated from the main peak. Nor could the sedimentation coefficient of fraction A_4 be determined. The boundary of this fraction appeared at first as a broad peak but thereafter quickly disappeared.

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Received October 7, 1965.